Genetically Engineering Yeast to Understand Molecular Modes of Speciation

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Abstract:

An understanding of the molecular mechanisms of speciation (reproductive isolation) is potentially very powerful as it should help us appreciate the processes that differentiate closely related organisms such as humans and chimpanzees. Currently, however, our understanding of such mechanisms is far from complete. The closely related yeast, *Saccharomyces cerevisiae* and *Saccharomyces mikatae,* represent ideal species to use to study molecular modes of speciation as the genomes of these of organisms have been sequenced [1] and are readily genetically manipulated. *S. cerevisiae* and *S. mikatae* are capable of mating, but the resulting hybrids are almost always sterile. Three molecular models often used to explain reproductive isolation include chromosomal rearrangements, simple sequence divergence upon which the mismatch repair system acts, and genetic incompatibility. In this proposal an outline of a genetic engineering based approach to characterize the roles of these mechanisms in the reproductive isolation of *S. cerevisiae* and *S. mikatae* is presented.

Introduction:

The *Saccharomyces 'sensu stricto'* group consists of six closely related yeast species (*S. cerevisiae, S. paradoxus, S. mikatae, S. bayanus, S. cariocanus* and *S. kudriavxevii*) that will mate but produce sterile hybrids[2]. Three mechanisms of post-zygotic reproductive isolation have been proposed to play a role in this observed hybrid sterility. The first of these mechanisms invokes chromosomal rearrangements such as translocations as the cause of hybrid sterility, as such rearrangements may drastically hinder the meiotic pairing of chromosomes thereby leading to chromosomal segregation problems[3]. This model is supported by the recent finding that when one the four reciprocal translocations in the genome of *S. cerevisiae* relative to that of *S. mikatae* was removed, the fraction of fertile *S. mikatae*/*S. cerevisiae* hybrids significantly increased[4]. The second model used to explain *'sensu stricto'* hybrid sterility is that of simple sequence divergence upon which the mismatch repair system acts. This model poses that the mismatch repair system through its participation in the process of anti-recombination reduces meiotic homologous recombination between diverged sequences and therefore leads to chromosomal missegregation and gamete inviability[5, 6]. The observation that the fertility of *S. paradoxus/S. cerevisae* hybrids increased when the mismatch repair genes *PMS1* and *MSH2* were deleted clearly supports this model[7, 8]. The third proposed mechanism of yeast post-zygotic isolation states that genetic incompatibilities reduce hybrid fertility. For example, certain alleles from one/both of the parent species may be incompatible when expressed in a hybrid genetic background. The observation that tetraploid *'sensu stricto'* hybrids display fertility comparable to their parent species likely rules out dominant genetic incompatibility as a mechanism of yeast reproductive isolation, but recessive incompatibilities that limit fertility are still quite possible[9].

The mechanisms described above are all hypothesized to play a role in post-zygotic reproductive isolation of specific *Saccharomyces 'sensu stricto'* yeast. Nevertheless, these mechanisms are sometimes treated as if they are mutually exclusive. In reality, however, it seems possible that some or all of these mechanisms can act in concert. Thus, in this proposal we outline a plan to study the interplay of these three

mechanisms in the reproductive isolation of the *Saccharomyces 'sensu stricto'* yeast, *S. cerevisiae* and *S. mikatae*.

Specific Aims:

Specific Aim 1:

To determine the fraction of hybrid sterility that can be explained by reciprocal translocations in the *S. cerevisiae* genome relative to that of *S. mikatae*.

Background/Rationale:

Although Delneri et al. demonstrated that by "co-linearizing" the *S. cerevisiae* genome to that of *S. mikatae* the fraction of viable hybrid spores produced can be increased, they lacked complete *S. mikatae* genomic sequence and therefore failed to consider three reciprocal translocations[1, 4]. Therefore, by completely co-linearizing the *S. cerevisiae* genome a more accurate estimate of the role of chromosomal rearrangements (i.e. reciprocal translocations) in *S. cerevisiae/S. mikatae* hybrid sterility will be obtained.

Experimental Approach:

To co-linearize *S. cerevisiae* genome to that of *S. mikatae* a modified version of the Cre/Loxp system used in [4] will be employed. Briefly, two Loxp sites flanking separate selectable markers will be inserted into the *S. cerevisiae* genome at two sites between which a translocation has occurred. After selecting for insertion, the popping out of the selectable markers and the creation of reciprocal translocations will be induced by transforming yeast with a plasmid containing Cre recombinase under an inducible promoter. Yeast will then be replica plated onto a medium that allows one to test for marker loss and diagnostic PCR/gel electrophoresis will be used to select the colonies containing the desired translocation. The Loxp sites will then be removed via plasmid mediated homologous recombination, and the above steps will be repeated to generate a strain containing all desired translocations.

To assay the fraction of hybrid sterility attributable to chromosomal translocations, haploid cells from the co-linearized *S. cerevisiae* strain will be mated to spores of *S. mikatae*. Sporulation will be induced and the number of viable spores will be determined. As a control, a wild-type *S. cerevisiae* strain also will then be crossed with *S. mikatae*. It seems likely that the observed fertility rate of the co-linearized hybrids will be significantly larger than that of the control. Standard statistical tests such as a χ^2-squared test can be used to assess the significance of observed differences.

Specific Aim 2:

To determine the fraction of *S. mikatae/ S. cerevisiae* sterility attributable to the mismatch repair system's inhibition of meiotic recombination.

Background/Rationale:

As mentioned above, the mismatch repair system likely acts as a barrier to inter-specific mating through its participation in anti-recombination. The role of the mismatch repair system in the post-zygotic isolation of *S. cerevisiae* and *S. mikatae*, however, has not been tested. Establishing that this system plays part in the isolation of these species would be significant as it would one of the first studies to suggest that both chromosomal rearrangements and simple sequence divergence upon which the mismatch repair system works simultaneously take part in post-zygotic reproductive isolation.

Experimental Approach:

S. mikatae and *S. cerev*isiae *pms1Δ* and *msh2Δ* (mismatch repair gene knockout) strains will be obtained from the deletion consortium or created via standard disruption protocols and the corresponding double mutants will be constructed[10]. The *S. mikatae* and *S. cerev*isiae *pms1Δmsh2Δ* strains will then be crossed and analyzed as described in specific aim 1. As a control, the sterility rate of wild-type *S. cerevisiae/S. mikatae* hybrids will also be assayed. Since the deletion of *PMS1* and *MSH2* may lead to the accumulation of mutations that are lethal in haploids, isogenic intra-specific crosses between wild type and *pms1Δmsh2Δ S. cerevisiae* and *S. mikatae* strains will be performed. The following conservative measure of spore viability will then be used: $F_{corrected}^{Cer/Mik} = F_{observed}^{Cer/Mik} + \min(F_{Wildtype}^{Cer/Cer} - F_{pms1\Delta msh2\Delta}^{Cer/Cer}, F_{Wildtype}^{Mik/Mik} - F_{pms1\Delta msh2\Delta}^{Mik/Mik})$ where F stands for the spore viability rate. If the mismatch repair system is in fact preventing meiotic recombination, the rate of spore viability in the double knockout strains should be greater than that of the wild-type inter-specific control. It is conceivable that chromosomal rearrangements may put an upper-bound on hybrid viability. Thus, it would also be interesting to perform these experiments using the co-linearized strain of specific aim 1.

Specific Aim 3:

To determine if specific *S. cerevisiae or S. mikatae* chromosomes are present in viable hybrid spores more often than would be expected by chance.

Background/Rationale:

The role of genetic incompatibility in hybrid sterility is difficult to directly assess due to the large number of possible genetic interactions. Therefore, a more indirect approach must be taken. One such method is to simply assess if *S. cerevisiae or S. mikatae* chromosomes are present in viable hybrid spores more often than would be expected by chance. The rationale behind taking such an approach is based upon the observation that nearly all viable *S. cerevisiae/S. mikatae* hybrid spores are aneuploid (contain multiple copies of certain chromosomes) [4, 7, 11]. The non-random distribution of such chromosomes may generate insight

into the role of genetic incompatibility in hybrid sterility, as these chromosomes may contain genes that are non-functional when present in a hybrid genetic background. For example, if two copies of a specific *S. cerevisiae* chromosome were found in the majority of the viable *S. cerevisae/S. mikatae* spores this may suggest that two copies of gene(s) present on this chromosome are needed for viability. Perhaps such genes are silenced on one copy of the chromosome, thereby leading to the requirement of an additional chromosomal copy for viability.

Experimental Approach:

To determine the karyotypes of *S. cerevisiae/S. mikatae* hybrids, the DNA from viable hybrid spores of the crosses described in specific aims 1 and 2 will be isolated, and diagnostic PCR (with primers specific to each of the *S. cerevisiae and S. mikatae* chromosomes) and gel electrophoresis will be performed as in [4]. By comparing the intensity of each band on the gel to that of a control band representing one copy of the chromosome (identically amplified) it will be possible to determine if 0,1, or >1 copies of this chromosome are present in the spore. The karyotypes of at least 500 spores from each type of cross will be analyzed. The cumulative binomial distribution will be used to determine if certain chromosomes are present in spores more often than would be expected by chance: $P\{X \geq k_i^s\} = \sum_{i=k_i^s}^{N} \binom{N}{i} \left(\frac{1}{2}\right)^N$ where N is the number of spores analyzed and k_i^s is the number of chromosome i ($i = 1...16$) of species s (*S. cerevisiae and S. mikatae*) observed in all viable spores. The above equation assumes that each chromosome from a species has a probability of ½ of being in a hybrid haploid spore. Based upon previous studies [7, 11] it seems likely that certain chromosomes will be present in viable spores more often than expected by chance. It would be interesting to examine the features of these chromosomes for things such as the presence of known silenced or species specific genes. It is worth noting that it could also be quite insightful to look for common karyotypic profiles amongst the karyotypes of surviving spores. By representing the karyotype of each viable spore as a vector and clustering these vectors it may be possible to identify such profiles.

Conclusions and Significance:

The three mechanisms of speciation that the discussed in this proposal are often associated with the isolation of specific organisms. While chromosomal rearrangements are typically linked to plants[12], the mismatch repair system (acting upon sequence divergence), and genetic incompatibility are typically associated with yeast/bacteria [7, 13, 14] and flies[15] respectively. It seems probable, however, that these different mechanisms can act in concert in a single species. By studying the roles of these three mechanisms in the reproductive isolation of the yeast, *S. cerevisiae* and *S. mikatae*, we hope to characterize the interplay between these modes of speciation. An understanding of such interplay will likely help us grasp the processes that differentiate more complex organisms such as humans and chimps.

References

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